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OPTICALLY INTEGRATED MICROFLUIDIC CYTOMETER FOR HIGH THROUGHPUT SCREENING OF PHOTOPHYSICAL PROPERTIES OFF CELLS OR PARTICLES

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Abstract

Fluorescence photobleaching is a complex phenomenon that depends on several experimental and environmental factors. In fluorescent proteins, photobleaching occurs by both reversible and irreversible pathways, both of which may limit photon output. Under single-molecule excitation conditions, fluorescent proterins emit 10-100x fewer photons than small-molecule fluorophores. To investigate these photophysics for diverse fluorescent protein sequences, we developed versatile microfluidic platform capable of measureing photobleaching on individual mammalian cells at high-throughput (>;20 cells/s) under a ranger of excitation intensities (0.1 kW- GW/cm2). By employing a series of spatially separated excitation beams, the irreversible component of photobleaching was isolated. A mixture of red fluorescent proteins was assayed, and mCherry was identifies ad the variant with the lowest degree of irreversible photobleaching.This technology complements existing methods for fluorescent protein analysis, thereby facilitating the development of next-generation fluorescent proteins for single-molecule research.

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References

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Status of Availability

This invention is available for licensing exclusively or non-exclusively in any field of use.

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